CARDIAC ISOFORM OF ALPHA - 2 – MACROGLOBULIN AS AN EARLY MARKER FOR CARDIAC INVOLVEMENT IN HIV / AIDS PATIENTS

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BRANCH I – GENERAL MEDICINE

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CERTIFICATE

This is to certify that this dissertation entitled **"CARDIAC ISOFORM OF ALPHA - 2 – MACROGLOBULIN AS AN EARLY MARKER FOR CARDIAC INVOLVEMENT IN HIV / AIDS PATIENTS"** submitted by **Dr.R. OMNATH** to The Tamil Nadu Dr.M.G.R. Medical University, Chennai is in partial fulfillment of the requirement for the award of M.D. degree Branch I (General Medicine) and is a bonafide research work carried out by him under direct supervision and guidance.

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DECLARATION

I, **Dr. R. OMNATH** solemnly declare that I carried out this work on **"CARDIAC ISOFORM OF ALPHA - 2 – MACROGLOBULIN AS AN EARLY MARKER FOR CARDIAC INVOLVEMENT IN HIV/AIDS PATIENTS"** at Department of General Medicine, Government Rajaji Hospital during the period of March 2005 – April 2006. I also declare this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any university, board either in India or abroad.

This is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulation for the M.D. in General Medicine Degree examination.

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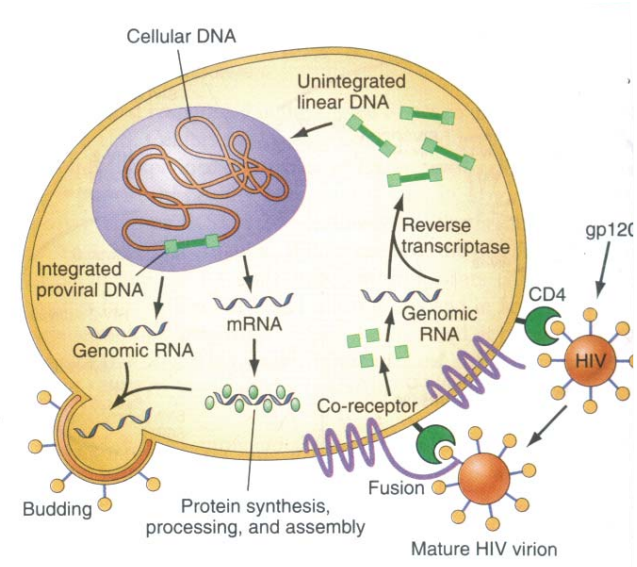
INTRODUCTION

The last three decades had witnessed the emergence of HIV infection as a global pandemic affecting approximately 42 million people worldwide[1]. Since its first recognition in 1983, the disease has been reported virtually from all corners of globe, spreading at a tremendous pace and this trend is gathering momentum despite various multifaceted containment strategies by health organizations. A report by Joint United Nations Program on HIV /AIDS in 2003 estimated that 5 million new cases are reported each year. 95 % reported cases occur in developing countries, with two- thirds of the cases from sub-Saharan Africa alone. In India alone it has been estimated that 5.1 million people are living with this illness. Today, AIDS alone account for a tragic death toll of 3 million deaths per year, making it the fourth leading cause of mortality worldwide[1].

HIV infection affects entire organ systems within the body. Both HIV – 1 and HIV – 2 belong to the family Retroviridae and the subfamily of lentiviruses. The infection is transmitted via various portals , sexual transmission either heterosexual or homosexual being the major route of transmission. Transmission via percutaneous needle exposure in intravenous drug abusers and health care personals is also on the rise. The virus gain entry into the host cell by attachment to the cellular receptor

CD4 molecule expressed on the surfaces of helper T-cells, monocytes / macrophages and dendritic / langerhans cells.

Diagram of HIV mode of infection and multiplication of HIV:



The replication cycle of HIV. See text for description. (*Adapted f*

Fauci, 1996.)

With in the host cell the viral RNA is converted into complementary DNA (c-DNA) by reverse transcriptase enzyme. The c-DNA gets incorporated into the host cell chromosomes. The integrated DNA is transcribed into messenger RNA (mRNA) and the viral proteins are synthesized within the host cell. These newly synthesized RNA and viral proteins are packaged together and released by the "budding process".

Average time period elapsed between infection and onset of AIDS is approximately 8- 10 years. Between this period the disease progress through a seroconversion and an asymptomatic phase of illness to primary generalized lymphadenopathy and a gamut of secondary infections and malignancies.

Table 1 : 1993 revised classification system for HIV infection and expanded AIDS surveillance case definition for adolescents and adults.

Classification

CD4 cell count (per micro liter)	**CLINICAL CONDITIONS**		
	A	**B**	**C**
	Asymptomatic acute primary infection or PGL	**Symptomatic but not A or C conditions**	**AIDS indicator conditions**
> 500	A1	B1	C1
200 - 499	A2	B2	C2
< 200	A3	B3	C3

HIV infected patients under classifications – A3, B3, C1 , C2 , C3 are defined as AIDS cases.

CD4 count < 200/µl is defined as AIDS regardless of presence of symptoms or opportunistic infections.

1993 AIDS surveillance case definition CDC Atlanta:

Category A:

One or more of the following in an adolescent (> 13 years of age) or adults as given in table 2:

Category B:

Consists of symptomatic conditions in an HIV infected adolescent or adult that are not included in clinical category C and that meets at least one of the following criteria.

a) The conditions are attributed to HIV infection or indicative of a defect in cell mediated immunity (CMI)
b) Conditions are considered by physician to have a clinical course or require management that is complicated by HIV infection

Category C: AIDS indicator conditions

Table 2: 1993 AIDS surveillance case definition CDC Atlanta

Category A 1 or more of the following :	**Category B:**	**Category C: (AIDS indicator conditions)**
i) Asymptomatic HIV infection ii) Progressive Generalized Lymphadenopathy iii) Acute primary HIV infection (history of acute HIV infection)	1.Bacillary angiomatosis 2.vulvo vaginal candidiasis , oral candidiasis 3.cervical dysplasia , cervical carcinoma in situ 4.constitutional symptoms > 1 month 5.oral hairy leukoplakia 6.herpes zoster - 2 distinct episodes or more than one dermatome 7.idiopathic thrombocytopenic purpura 8.listeriosis 9.pelvic inflammatory disease 10.peripheral neuropathy	1.Candidiasis of the bronchi , trachea or lungs 2.esophageal candidiasis 3.cervical cancer – invasive 4.coccidiomycosis – disseminated or extra pulmonary 5.cryptococcosis - extra pulmonary 6.cryptosporidiosis – chronic intestinal (> one month duration) 7.cytomegalovirus disease (other than lung , spleen and nodes) 8.CMV retinitis 9.HIV related encephalopathy 10.herpes simplex – chronic ulcer , bronchitis, pneumonia or esophagitis 11.histoplasmosis – disseminated or extra pulmonary 12. isosporiasis – chronic intestinal 13.kaposi ’s sarcoma 14.Burkett ’s lymphoma 15.immunoblastic lymphoma 16.primary brain lymphoma

		17.MAIC, M. kansasi infection - (disseminated or extra pulmonary) 18.M . tuberculosis – any site 19.mycobacterium other species or unidentified species -- (disseminated or extra pulmonary) 20.pneumocystis jiroveci pneumonia 21.recurrent pneumonia 22.progressive multifocal leukoencephalopathy 23.salmonella septicemia - recurrent 24.toxoplasmosis of brain 25.wasting syndrome

From the beginning of the epidemic it was recognized that the cardiovascular system could also be involved by the virus, but until recently this aspect was not adequately emphasized. Till late 1990s opportunistic infections and lymphoreticular malignancies were the principal mortality deciding factors in AIDS. With the advent of HAART there was a change in this scenario with dramatic improvement in life expectancy of HIV infected patients. As the survival was prolonged, long

term follow up of these patients revealed significant involvement of cardiovascular system both by the virus and as an adverse effect of HAART therapy[2- 7] contributing for morbidity and mortality in them. Hence it is contemplated that strategies focusing on early detection of these cardiovascular involvement in HIV patients should be an integral part of the comprehensive management of AIDS patients. Currently the main diagnostic tool available that can be utilized is echocardiography, which may warrant the need for specialized practitioners to be involved in management of every single case. Since this is difficult and cost-ineffective, the identification of a molecular biomarker correlating with cardiovascular involvement would be a novel effort.

Hitherto, available markers for monitoring HIV progression are CD4 T cell counts and HIV-RNA levels. But studies have revealed that they do not correlate with many forms of cardiac involvement including pericardial effusion and pulmonary hypertension.[2-7,22] Similarly no available cardiac biomarkers like CK-MB, troponins- T and I, myoglobin etc have been found to be elevated uniformly irrespective of the form of cardiac involvement. Recently Brain natriuretic peptide (BNP) has been found to correlate inversely with left ventricular ejection fraction and is now employed in some centers for distinction of congestive cardiomyopathy from other causes of dyspnoea[8]. But large scale studies

comparing its utility in HIV patients and in other forms of cardiac pathologies are still lacking.

Cardiac isoform of alpha-2-macroglobulin (CA2M) is a novel molecular marker that has been confirmed by a couple of recent studies to be such a marker [9-16] , the levels of which significantly raises during any form of cardiac illness irrespective of the nature of primary pathology. Hence the present study has been undertaken to explore the possibility of utilizing CA2M as a marker for cardiac involvement in HIV patients.

Aims & Objectives of the study

i. To estimate the levels of CA2M in HIV infected patients with and without cardiac involvement as detected on echocardiography .

ii. To assess the correlation of CA2M levels with cardiac involvement

Review of literature

HIV and cardiovascular involvement

The 2- to 5-year incidence of symptomatic HIV-related heart failure ranges from 4 to 28 percent suggesting a prevalence of 4 to 5 million cases worldwide. Among HIV infected children upto 10 years of age 25% die with chronic heart disease[2-5]. In a large autopsy series conducted by Barbero et al [18] in 440 patients cardiac involvement in HIV was documented in 18.6 % cases . The clinical implication of this remains still under evaluation, though several studies have concluded cardiac involvement to be an independent prognostic factor in morbidity and mortality . The exact mechanism by which HIV can infect CD4+ receptor negative cardiac myocytes still remains unclear but it has been postulated based on autopsy studies[18] that HIV can cause cardiac damage both by direct myocyte interaction mediated by reservoir cells (eg. dendritic cells) and indirect toxicity resulting from activation of multifunctional cytokines that contribute to progressive and late tissue damage. The major cardiovascular disorders documented in AIDS patients include left ventricular systolic dysfunction and congestive heart failure resulting from dilated cardiomyopathy , isolated right ventricular dysfunction , pericardial effusion, myocarditis, primary pulmonary hypertension, malignancies and accelerated

atherosclerosis [2-7,18-21] . In an echocardiographic analysis of 127 HIV patients, Cecchi et al.,[4] demonstrated that 92 patients had evidence of cardiac involvement with a high incidence of pericardial effusion (29%) and dilated cardiomyopathy (26%).

Dilated cardiomyopathy and Left ventricular systolic dysfunction

A 4 year observational study of 296 patients with HIV infection by Currie PF et al., revealed that 15% have dilated cardiomyopathy, 13 % isolated right ventricular dysfunction and 12 % with borderline left ventricular dysfunction[5] . Postulated mechanisms for HIV – mediated cardiomyopathy are[18-21] :

1. Direct cardiotoxicity of HIV virus
2. Opportunistic infections
3. Autoimmune response to viral infection
4. Cytokine alteration
5. Nutritional deficiencies- selenium
6. Drug toxicity

Though exact mechanism remains elusive majority consider it to be result of postmyocarditis cardiomyopathy. Idiopathic lymphocytic myocarditis is a common postmortem finding in patients with ventricular dysfunction The overexpression of cytokines TNF-α , IL s and inducible nitric oxide synthase (INOS) evident in endomyocardial biopsies appears

to be equally responsible . Occurrence of cardiomyopathy in children , in whom a disease unrelated to HIV infection would be rare , suggests a direct relationship between HIV disease and cardiomyopathy[3]. Presence of cardiomyopathy correlate with low CD4+ counts and is a grave prognostic sign[18-21].

Pericardial effusion

Pericarditis and pericardial effusion constitute most common recognized cardiac involvement in HIV infection. In a review of 15 autopsy and echocardiographic series involving 1139 patients with HIV disease, Heidenreich et al. observed that, incidence of pericardial disease was 21%[22]. Factors causing pericardial effusion include opportunistic infections , most common being mycobacterium tuberculosis or MAI infection , direct viral toxicity and malignancies – Kaposi's sarcoma and lymphoma. It may be part of a generalized capillary leak syndrome due to over production of inflammatory cytokines [23]. Others are uremia and drug toxicity . Occurrence of pericardial effusion is independent of CD4+ count[6] and is a grim prognostic sign with 6 month survival rate of merely 36%[22], thus denoting an end-stage disease.

Pulmonary hypertension

In 2 independent analysis of hospitalized AIDS patients Seonin et al. and Saidi et al. observed that, primary pulmonary hypertension occur in about 0.5% of [24,25]. Lung histology frequently demonstrates plexogenic arteriopathy as in other cases of primary pulmonary hypertension. It is often considered to develop secondary to recurrent lung infections, venous thromboembolism, or left ventricular dysfunction, but its occurrence in HIV-infected patients without documentation of prior history of any of these risk factors indicate some hitherto elusive mechanisms also contribute. Hypothesized factors are cytokine related stimulation and proliferation of endothelium and smooth muscle hyperplasia due to receptor mediated action of viral proteins[23-27]. In a review of 88 reported cases of pulmonary hypertension in AIDS patients Mesa et al. found no correlation of pulmonary hypertension with CD4+ count[27].

Ventricular diastolic dysfunction

Left ventricular diastolic dysfunction accompanied by left ventricular hypertrophy is more pronounced in AIDS patients[27-30]. Nishimura et al reported presence of left ventricular diastolic dysfunction in 53.8% of 40 asymptomatic AIDS patients with a normal ejection fraction[31]. Left ventricular wall thickness and mass index tend to be

increased among AIDS patients compared to normal subjects. The pathogenesis of diastolic dysfunction is not clearly defined. Possible etiologies include direct infection of heart by HIV virus and opportunistic infections (myc. avium, cryptococcus neoformans, toxoplasma gondii) or direct involvement by a neoplastic process (kaposi sarcoma, lymphoma) and infiltrative diseases (amyloidosis, tuberculosis).

Infective endocarditis

Though intravenous drug abuse may be contributory to increased incidence of endocarditis, incidence of endocarditis in HIV infected patients is comparable to that of non-HIV infected individuals[31]. Common pathogens are Staphylococcus aureus and Salmonella . Fungal endocarditis is caused by Aspergillus fumigatus , Candida and Cryptococcus neoformans and usually accompanies systemic fungemia. It will cause a rapid downhill deterioration in the illness already compromised by cachexia and defective immunity .Besides infective causes 3-5% of AIDS patients ,mostly those with wasting syndrome develop marantic endocarditis

Accelerated atherosclerosis and other vascular involvement

An increased risk for premature atherosclerotic diseases is noted in HIV patients on treatment with protease inhibitors even in the absence of

other established risk factors like hypertension , diabetes mellitus etc. Insulin resistance and lipodystrophy are well known adverse effects of therapy with protease inhibitors[32-34] This effect is due to impaired hepatic chylomicron uptake and endothelial triglyceride clearance coupled with increased apoptosis of adipocytes culminating in hyperlipidemia and insulin resistance. Abnormalities in lipid profile have also been noted with usage of efavirenz.

Besides this a wide range of inflammatory vascular diseases including polyarteritis nodosa ,Henoch-Schonlein purpura and drug induced hypersensitivity vasculitis may develop in HIV infected patients. Also HIV patients are at a higher risk for development of hypertension at a younger age than the general population due to HIV induced endothelial dysfunction, vasculitis and accelerated atherosclerosis impairing renal blood flow.

Cardiovascular malignancy

Kaposi's sarcoma and less frequently malignant lymphoma occur in AIDS patients[27,35]. Cardiac involvement in Kaposi's sarcoma indicate a disseminated disease. Cardiac Kaposi's sarcoma is usually not obstructive or associated with clinical cardiac dysfunction, morbidity or mortality. Lymphomatous infiltration may be diffuse or may result in

discrete isolated lesions, which are usually derived from the Burkitt or immunoblastic type B cells .

Other abnormalities which have been detected include autonomic dysfunction, arrhythmias and isolated right ventricular dysfunction[27].

Current guidelines for workup

Currently only patients having symptoms and signs with regards to cardiovascular involvement such as shortness of breath, pericardial rub, S3 gallop or other signs of congestive failure are subjected to detailed evaluation with echocardiography in accordance with the previous concept that sub clinical cardiac illness do not influence the course of the illness[6].

Nevertheless, in light of the recent studies which strongly stress on focusing on cardiac involvement for better treatment outcome, this approach need to be modified so that timely detection of events can be done. Since frequent echocardiography by an expert may not prove cost - effective a molecular biomarker which has a high sensitivity and specificity for cardiac illness would be a novel one.

A review of the available molecular markers

Currently available markers for cardiac illness are CK -MB, Troponin I / T , myoglobin and BNP (Brain Natriuretic Peptide).

Though widely used each one has limitations and drawbacks. CK-MB, cardiac isoform of creatine kinase is the most widely used marker for diagnosing myocardial infarction . Though specificity is high, false positive results may be obtained in myopathies , renal failure , hypothyroidism etc . Similarly diagnostic value is limited only to cases of myocardial necrosis[36-37] . The same applies to cardiac troponins as well, though their sensitivity in diagnosing minimal injuries to the heart is high compared to CK-MB . Myoglobin is useful only for diagnosis of myocardial infarction in the first one or two hours when other markers may not be elevated . BNP is a natriuretic peptide synthesized by ventricular myocardium in response to elevation in ventricular end diastolic pressure and volume . It has been approved by FDA as a diagnostic test for differentiating cardiac and respiratory causes acute dyspnea. An elevated levels of BNP is diagnostic of acute left ventricular failure .It is also considered to be a prognostic marker for chronic heart failure and in acute coronary syndromes[38-40]. Diagnostic utilities of BNP and its precursor form NT-pro BNP, which is more stable and has a better half life and serum concentrations , are still under large scale trials so that a universal consensus regarding its usage can be formulated .

Cardiac isoform of Alpha 2 Macroglobulin (CA2M)

Alpha 2 Macroglobulin (α2M), a plasma glycoprotein, is an acute phase reactant synthesized by liver . In 1994, Rajamanickam et al, isolated a cardiac isoform of Alpha 2 Macroglobulin (CA2M) from sera of aorta – constricted rats which was found to play a role in mediating cardiac hypertrophy(Genbank accession nos AY9196111 , AY921651 and AY887133) . Biochemical characterizations found that this protein with a molecular weight 182 kDa is similar to α2M with respect to molecular weight and biochemical properties. This had been postulated to act as a molecular trigger signaling several molecular events associated with the induction of cardiac hypertrophy under pressure overloaded stress conditions[9,10].This was confirmed by the observation that cDNA of 182 kDa serum protein, isolated from hypertrophied cardiac myocytes in aorta constricted animals, induced hypertrophy upon direct injection in rat hearts[12]. The protein being synthesized in heart cells could have an autocrine or paracrine role in the development of cardiac hypertrophy[9,10]. The distinction between these two similar serum proteins, *i.e.*, α2M and CA2M (182 kDa), is evident from the fact that the anti-human α2M antibody did not cross-react with the 182 kDa protein whereas the anti-182 kDa protein antibody did cross-react with both rat and human α2M.Also, the isoelectric point of 182 kDa protein passes into

the value of α2M PI but with a lower number of protein spots (3 spots) than α2M (4 spots). In addition, purified α2M protein also failed to induce cardiac hypertrophy in experimental animals. Studies on the localization of 182 kDa protein induced during the development of cardiac hypertrophy showed that the 182 kDa protein was found in the cytosolic fraction obtained from the hypertrophy- induced heart by aortic constriction but not in the liver.

Physiochemical properties

Biochemical characterizations suggest that the 182 kDa protein shares maximal homology with α2 macroglobulin (α2M) Both the proteins belong to the glycoprotein family. The molecular weight of α2M is 725,000, as determined by ultracentrifugation, estimates of partial specific volume range between 0.72 and 0.735, and the isoelectric point 5.0-5.2. α2M is a glycoprotein containing 8-10% carbohydrate (hexose 4.25%, hexosamine 3.4%, fucose 0.2% and sialic acid 2.0%).The native α2M protein exist in two quite distinct forms: the electrophoretically "slow" S form, which is the circulating form that is reactive with proteinases; and the electrophoretically "fast" F form, which is produced irreversibly from the S form by reaction with proteinases or low-molecular-weight amines. α2M consists of four identical subunits that are disulfide bonded in pairs, and the half-molecules thus formed are

associated noncovalently. The subunit contains a bond about two-thirds of the way from the N-terminus that tends to be cleaved spontaneously in denatured S-α2M, especially at elevated temperatures and alkaline pH[41].

α2M receptor

The low-density lipoprotein receptor-related protein (LRP) has been identified as a α2M receptor[42]. It is a 600-kDa endocytic membrane-bound receptor belonging to the low-density lipoprotein family and is expressed in a broad spectrum of cell types as a 4525-amino acid residue single chain precursor. It is processed into a 85 kDa transmembrane β chain and an approximately 515 kDa α chain non-covalently associated with the extracellular part of the β chain.

Reaction of α2M with proteinases causes a major conformational change in the protein that physically entraps the proteinase and also exposes the receptor recognition sites on each of the four identical subunits of the protein. The receptor-binding domain identifies only the activated α2M complexes and does not bind the native α2M[43]. Binding to the multivalent LRP facilitates endocytosis of the ligand-receptor complex and clearance of α2M from the cell surface. Binding also elicits increases in intracellular inositol triphosphate, calcium, and cyclic AMP levels and also activates protein kinase C and phospholipase C -gamma and promotes alkalinization of cell cytoplasm[44]. Protein phosphorylation

is a fundamental mechanism whereby a variety of extracellular stimuli modulate cellular function. The serine-threonine kinase and protein kinase C (PKC) is strongly implicated in the cardiac hypertrophic response. It has also been shown that, the signaling pathways of stretch induced cardiac hypertrophy are mostly through the activation of PKC45. Both the aorta constricted as well as the 182 kDa protein (CA2M) injected rats showed up to a 3 fold induction of PKC activity within 10 minutes of stress imposition.

Cardiac gene transfer has been previously achieved predominantly by direct injection into the myocardium or perfusion of an isolated coronary segment. Either of these approaches result in focal overexpression of the transgene and is therefore unlikely to modulate global cardiac function effectively.To overcome this problem the full-length cDNA was cloned along with the signal peptide, thus rendering the 182 kDa protein that is synthesized to be secretory so that the protein is released into the circulation and is able to exert its effect, binding to many growth modulating factors in the serum, and then targeted to cardiomyocytes by a receptor mediated mechanism[10]. The expressed protein of cDNA of 182 kDa is able to induce cardiac hypertrophy is evinced by the induced expression of the muscle specific marker genes namely β-MHC, MLC-2 and ANF characteristic of pressure overloaded

cardiac hypertrophy. Recent studies also clearly demonstrates and confirms the induction of β-MHC and the c-fos gene upon p182-pcDNA3.1 (-) injection, characteristic of pressure overloaded cardiac hypertrophy[12].

Clinical utility of CA2M

The prospective role of CA2M as a molecular marker for cardiac dysfunction in various forms of cardiac diseases has been analyzed in a couple of recent trials . Dr Ratinavel et al[13] reported that levels of CA2M is significantly higher in patients having cardiac dysfunction resulting from various forms of cardiac illnesses including rheumatic heart disease, ischemic heart disease and congenital heart diseases. In his study the serum levels of CA2M was significantly high. (131$\pm$27.14mg/dl) when compared to values in non-cardiac patients (47$\pm$5.2). In another study Annapoorani et al[14] demonstrated that CA2M level is elevated in post myocardial infarction in diabetic patients. She concluded that CA2M levels could be used as a marker for retrospective identification of silent myocardial infection in diabetic patients. It was also observed that values of CA2M in post-myocardial infarction patients without diabetes were also elevated in parallel, stressing cardiac dysfunction specificity of this protein. These studies elucidated the potential role of CA2M as a cardiac

biomarker which correlates with the cardiac involvement by various diseases irrespective of primary pathologic process.

Materials and methods

This study was conducted at Government Rajaji hospital, Madurai in collaboration with Department of Biochemistry, Madurai Kamaraj University . HIV infected patients involved in the study were selected from ART clinic, Government Rajaji Hospital . CA2M estimation in subjects and controls using ELISA and Western blot analysis were done at Laboratory, Department of Biochemistry, Madurai Kamaraj University. The study was approved by Ethical committee, Government Rajaji Hospital. An informed consent was obtained from all study participants.

Nature of Study : Analytical Study,

Sample Size - 89

Sample selection

i. Inclusion criteria

69 HIV infected patients with symptoms suggestive of cardiovascular involvement in accordance to a standard questionnaire (see Appendix) were selected from ART clinic after preliminary clinical assessment .Basic investigations were performed before subjecting to detailed cardiovascular system evaluation by means of chest X-ray , Electrocardiography and Echocardiography .At the end of workup they

were divided into two groups – those with cardiovascular manifestations of HIV and those without.

ii) Exclusion criteria

Since the aim of the study is to analyze the usefulness of CA2M as an early marker for cardiovascular manifestations of HIV/AIDS , patients with clinical features of NYHA class III / IV cardiac failure and patients who had signs of congestive cardiac failure like S3 gallop, elevated JVP and lung basal crepitations were not included in the study. Similarly patients with known history of cardiac illnesses like ischemic heart disease, rheumatic heart disease, congenital heart disease etc were not included .

Patient categorization

After selecting the desired study population , each patient was subjected to clinical examination and subsequently investigations were done according to the proforma. Categorization into two groups was done based on evidence of cardiovascular involvement by echocardiography – Group A comprising of patients having echocardiographic findings and GroupB of patients who did not have any echocardiographic abnormalities.

For comparison of results to a non-HIV infected population , two groups of age and sex matched individuals ,each having 10 members –

Group C comprising of patients with various cardiac diseases (ischemic heart disease , rheumatic heart disease , congenital heart disease) and Group D comprising of normal individuals , were also included in the study .

Echocardiography

M-mode ,2-D and Doppler echocardiographic studies were recorded for each patient.

The abnormalities were defined as:

1. Dilated cardiomyopathy – left ventricular dilatation and global systolic dysfunction Left ventricular ejection fraction is calculated by the formula :

   EF= (EDV-ESV)/EDV x 100 % EDV- End diastolic volume, ESV- End systolic volume .

   Normal values of LVEF- 55- 75 %

   < 45% was considered abnormal.

2. Diastolic dysfunction-

   Restrictive filling pattern of left ventricle is determined by the ratio of early to late filling velocity by Doppler (E/A ratio)

   Normal mitral E/A ratio is – for adults<41 years 2.1±0.6 and for adults >55years 1.3 ± 0.3. Values above the limit are considered abnormal.

3. Pulmonary hypertension -

   Estimation of the pulmonary artery systolic pressure is derived using Bernoulli's equation using tricuspid regurgitant jet .A fixed value of right atrial pressure - 5 or 10 mmHg, was added to the transtricuspid pressure gradient to yield systolic right ventricular pressure (SRVP).

   In the absence of pulmonic stenosis SRVP is approximated to be systolic pulmonary artery pressure (SPAP).

   Pulmonary hypertension is defined as SPAP > 30mm Hg at rest.

   Severe pulmonary hypertension is SPAP > 50mm Hg at rest.

4. Mitral regurgitation – Severity is graded as:

   Grade I – jet (%LA) <15 and faint spectral doppler flow and ERO(effective regurgitant orifice) <0.2 cm^2

   Grade II- ERO 0.2 to 0.29 cm^2

   Grade III- ERO 0.3 to 0.39 cm^2

   Grade IV- – jet (%LA) >50 and dense spectral doppler flow and ERO >0.40 cm^2

5. Aortic regurgitation – Severity is graded as:

   Grade I- jet height(%LVOT) <25 , faint spectral doppler flow and pressure half time (msec) >400

   Grade II- jet height(%LVOT) 25-46

Grade III- jet height(%LVOT) 47-64

Grade IV- jet height(%LVOT) >65, dense spectral doppler flow and Pressure half time (msec) <250

6. Pericardial effusion- defined as an echo-free space surrounding heart. Quantified as minimal ,mild ,moderate or large.

   Minimal – 5-20 ml fluid in pericardial cavity

   Mild- <5 mm in maximum dimension

   Moderate – 15-20mm in maximum dimension

   Large - >20 mm maximum dimension

Collection of sera for CA2M estimation

The serum samples were collected in sterile EDTA coated tubes and within 5 hrs the samples were subjected to centrifugation for 15 min and then stored at -70º C until use. No samples were frozen/thawed more than twice.

Raising CA2M antibodies in rats

a) Pressure overload cardiac hypertrophy in Young Wistar albino rat was induced using aortic banding tantalum hemoclip

b) Purification of 182-kDa protein: The 182-kDa protein from the blood serum of aorta-constricted rats was purified by the method of Marriappan et al [9]. Total serum proteins were subjected to gel

chromatography on a Affi-Gel blue column and eluted in DEAE-Sepharose column .The eluted fractions from DEAE-Sepharose column containing the 182-kDa protein were pooled and fractionated in the BIOPAD HPLC system using a gel filtration column .The fraction containing the 182-kDa protein was collected and stored at –70°C.

c) Raising of anti-rat 182-kDa antisera

Experimental rabbits were injected subcutaneously with 500 µg of HPLC purified 182-kDa protein along with Freund's complete adjuvant. After 3 weeks, a booster injection of animals of about 200µg of 180 kDa protein with incomplete Freund's adjutant was given. In the third week following the booster dose, the rabbit was bled by an intra cardiac puncture and serum was separated and further fractionated in ammonium sulphate [50% saturation] . The ammonium sulphate fractionation wais then suspended in PBS, dialyzed against PBS for 12 hr and aliquots stored at –70ºC .

Western blot analysis of patient's sera with rabbit anti-rat cardiac A2M sera:

Immuno-cross reactivity between human sera and rabbit anti-rat CA2M sera was carried out by western blot analysis as detailed by Prabhakar and Rajamanickam . Briefly, 200mg of the total patients serum was fractionated on 10% SDS-polyacrylamide gel by electrophoresis .

The proteins were then transferred onto a nitrocellulose membrane. After transfer, treatment with antibody sera was carried out at 1:500 ratio at room temperature for 2h. Then a second antibody treatment was done with a 1:1000 ratio horseradish peroxidase- [HRP] conjugated goat anti-rabbit IgG at room temperature for 3h for labeling . The filter was washed and developed with 4- Chloronaphthol substrate solution [0.006% 4-Chloronaphtal, 0.0002% of 30% H_2O_2 at 20% methanol in PBS]

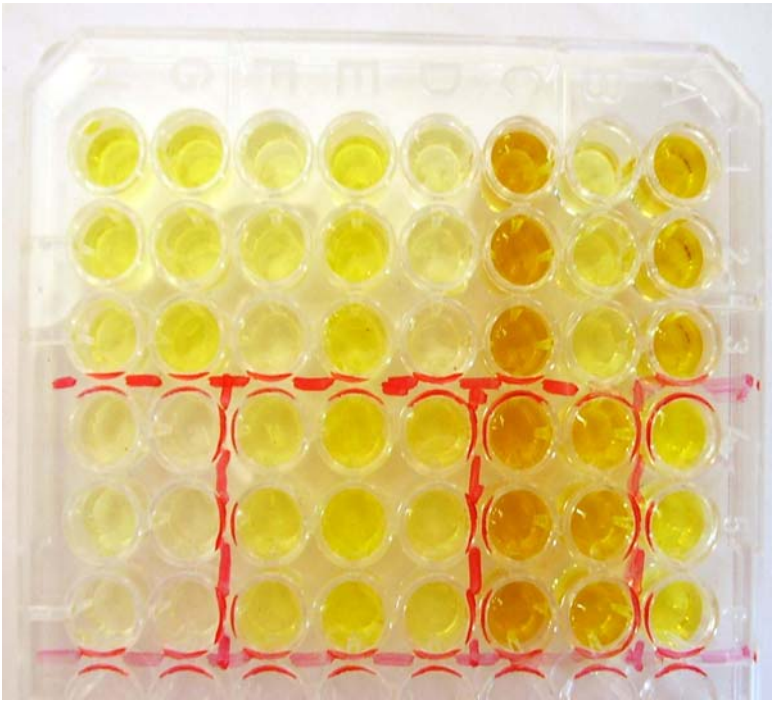


Fig 1 : Western blot analysis of patient's sera

with rabbit anti-rat CA2M antisera

CA2M measurements by Sandwich ELISA :

CA2M levels serum in the sera of normal human and patients with cardiac and non-cardiac diseases were quantified by sandwich ELISA using anti-rat CA2M antibody raised in rabbit . The optimal concentration of antigens and antibody for coating was determined by checkerboard titration's [BIORAD model 450 micro plate reader, Bio-Rad Laboratories, Hercules California, USA].

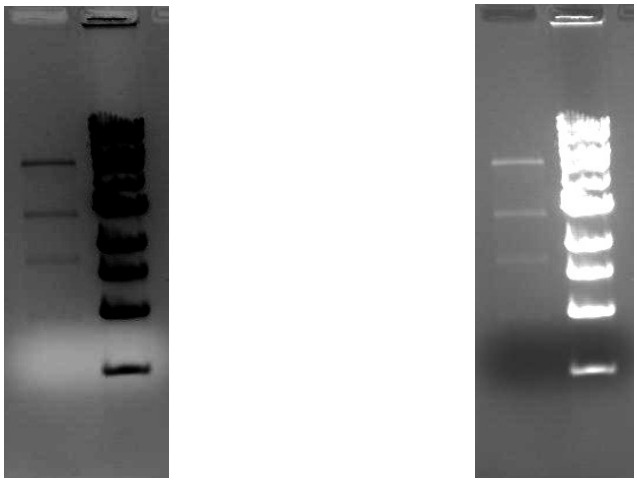


Fig 2 : Sandwich ELISA of CA2M antigen and anti-rat CA2M antibody

RESULTS

Computer Analysis of Statistical data was done utilizing Epidemiological Information Package (EPI 2005) developed by World Health Organization. Frequencies, percentages, range, median, mean, S.D. and ‘p’ values were calculated using this package.

STATISTICS

A. GENERAL CHARACTERISTICS

Table 1:Age characteristics of study population

Age Group	Group A (HIV With Cardiac inv (Echo +ve) (n=31)		Group B (HIVWithout Cardiac inv) (ECHO-ve) (n=38)		Group C (Cardiac without HIV)		Group D (Normal Healthy)	
	No.	**%**	**No.**	**%**	**No**	**%**	**No**	**%**
≤20	-	-	-	-	1	10	1	10
21-30	12	38.7	17	44.7	3	30	3	30
31-40	14	45.2	19	50	2	20	6	60
>40	5	16.1	2	5.3	4	40	-	-
Total	31	100	38	100	10	100	10	100
Mean	33.1 yrs		31.7 yrs		35.7 yrs		32 yrs	
S.D.	6.2		5.7		10.3		7.1	
P	0.5365							

Mean age of HIV population having cardiac involvement was 33.1±6.2 years and for those who did not have was 31.7±5.7 years. For the control group patients with cardiac diseases had a mean age of 35.7±10.3 years and normal healthy population had a mean age of 32±7.1 years. There is no statistically significant difference in the age composition between the study groups.

Table 2: Sex composition of study groups

Sex	Group A (HIV With Cardiac inv (Echo +ve) (n=31)		Group B (HIVWithout Cardiac inv) (ECHO-ve) (n=38)		Group C (Cardiac without HIV)		Group D (Normal Healthy)	
	No.	%	No.	%	No	%	No	%
Male	19	61.3	20	52.6	6	60	6	60
Female	12	38.7	18	47.4	4	40	4	40
P	0.5577							

Statistically significant difference does not exist in the sex composition between the study groups. All the groups were comparable with respect to sex distribution.

Table 3: CA2M Levels (mg/dl)

CA2M (mg/dl)	Group A (HIV with Cardiac inv)	Group B (HIV without Cardiac inv)	Group C (Cardiac without HIV)	Group D (Normal Healthy)
Range	42.57-128.6	37.3-59.65	110.38-140.28	32.46-42.18
Median	97.42	43.68	125.29	36.68
Mean	98.99	45.6	125.46	36.94
S.D.	23.7	5.8	9.07	2.96
P	0.0001			

- Normal healthy cases have lowest CA2M Values (36.94±2.96mg/dl)
- HIV without cardiac involvement have lower CA2M Values (45.6±5.896mg/dl)
- HIV with cardiac involvement have higher CA2M Values (98.99±23.7mg/dl)

There is statistically significant difference between HIV patients having cardiac involvement when compared to HIV patients without any cardiac involvement.

B. Characteristics of Group A and B (HIV population)

Table 4

VARIABLE	Group A HIV with cardiac (Echo +ve) (n=31)		Group B HIV without cardiac (Echo –ve) (n=38)		Total (n-69)		P
	No	%	No	%	No	%	
a) Sex Male Female	 19 12	 61.3 38.7	 20 18	 52.6 47.4	 39 30	 56.5 43.5	 0.6329 (Not Significant)
b) ART Treatment ZLN SLN Nil	 15 15 1	 48.4 48.4 3.2	 20 18 -	 52.6 47.4 -	 35 33 1	 50.7 47.8 1.4	 0.9134 (Not Significant)
c) Pulmonary TB + ve - ve	 10 21	 32.3 67.7	 19 19	 50 50	 29 40	 42 58	 0.215(Not significant)
d)Pericardial Effusion	10	32.4	-	-	10	14.5	-
e) Dilated Cardiomyopathy	4	12.9	-	-	4	5.8	-
f) Pulmonary Hypertension	15	48.5	-	-	15	21.7	-
g) Other forms	11	35.5	-	-	11	15.9	-

In group A, echocardiography revealed the following data :

Pericardial effusion- 14.5%, Dilated cardiomyopathy- 5.8%, Pulmonary Hypertension- 21.7%. 8 patients had more than one lesion. Mitral valve prolapse was present in 4 patients, 2 patients had LV diastolic dysfunction and 2 patients had Aortic regurgitation.

Table 5

VARIABLE	Group A HIV with cardiac (Echo +ve) (n=31)		Group B HIV without cardiac (Echo –ve) (n=38)		Total (n-69)		P
	Mean	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	
Age	33.1	6.2	31.7	5.7	32.3	5.9	0.3807
Duration of illness	2.58	1.72	2.33	1.92	2.44	1.82	0.3361
CD4 count	110.5	63.3	138.8	47.3	126.12	56.42	0.0097
CA2M Values	98.99	23.73	45.62	5.77	69.6	31.33	0.0001 significant

C.RELATIONSHIP OF VARIOUS VARIABLES WITH CA2M LEVELS IN GROUPS A and B

Table 6

Age and CA2M Values

Age Group	Cases		CA2M Values			
	No.	%	Range	Median	Mean	S.D.
≤20	-	-	-	-	-	-
21-30	29	42	38.4-120.34	49.86	63.48	27.7
31-40	33	47.8	37.3-128.01	51.46	69.52	31.4
>40	7	10.1	40.46-128.6	97.42	95.31	36.57
Total	69	100	37.3-128.6	51.46	69.6	31.3
P	0.0752					

CA2M Values are not significantly affected by age.

Table 7

Sex and CA2M Values

Sex	Cases		CA2M Values			
	No.	**%**	**Range**	**Median**	**Mean**	**S.D.**
Male	39	56.5	40.42-128.6	52.36	74.17	33.87
Female	30	43.5	37.3-120.4	50.21	63.65	27.09
P	0.181					

The CA2M Values are not significantly affected by sex.

Table 8

CD4 Count and CA2M Values

CD4 Count	Cases		CA2M Values			
	No.	%	Range	Median	Mean	S.D.
Above 200/mm^3	8	11.6	37.3-128.6	48.42	60.25	24.88
Below 200/mm^3	61	88.4	39.3-106.02	51.46	70.82	32.05
P	0.3026					

CD4 < 200/ mm^3 is defined as AIDS irrespective of symptoms and signs Though mean CA2M levels for CD4 Count <200 was 60.25±24.88 mg/dl and CD4 Count >200 was 70.82±32.05 mg/dl the difference was not statistically significant.

Table 9

Duration of HIV Illness (in years) and CA2M Values

Duration of HIV Illness	Cases		CA2M Values			
	No.	%	Range	Median	Mean	S.D.
≤ 1 year	22	31.9	37.3-128.6	46.6	69.2	33.7
1.1-3 yrs	33	47.8	38.4-128.49	50.4	63.8	27.6
3.1-5 yrs	9	13	42.08-124.01	96.2	92.9	31.1
> 5 yrs	5	7.2	40.31-126.21	54	67.6	34.7
p	0.2996					

There is no statistically significant relation between duration of HIV Illness and CA2M Values. But since duration of illness was defined since the time of detection of AIDS, the temporal association between disease progression and CA2M levels could not be conclusively commented by this single observation.

Table 10

ART and CA2M Values

ART	Cases		CA2M Values			
	No.	**%**	**Range**	**Median**	**Mean**	**S.D.**
ZLN	35	50.7	37.3-128.6	52.36	69.48	32.32
SLN	33	47.8	38.4-128.01	50.36	68.24	30.01
Nil	1	1.4	118.24	118.24	118.24	-
P	0.4392					

CA2M values are not significantly affected by type of ART treatment given. As the study was not a prospective analysis, this single observation cannot be regarded as significant.

Table 11

History of PT and CA2M Values

History of PT	Cases		CA2M Values			
	No.	**%**	**Range**	**Median**	**Mean**	**S.D.**
Positive	29	42	37.3-	50.36	65.7	29.22
Negative	40	58	38.4-	56.1	72.42	32.85
p	0.8601					

History of PT does not affect CA2M Values.

Table 12

Pericardial effusion and CA2M Values

History of PE	Cases		CA2M Values			
	No.	%	Range	Median	Mean	S.D.
Present	10	14.5	92.38-128.01	122.18	114.73	14.7
Absent	59	85.5	37.3-128.6	49.38	61.95	26.58
p	0.0001					

Among cardiac cases with HIV, patients having pericardial effusion have higher CA2M values than those without. This difference is statistically significant.

Table 13

Dilated cardiomyopathy and CA2M Values

H/O DCM	Cases		CA2M Values			
	No.	%	Range	Median	Mean	S.D.
Yes	4	5.8	91.34-118.26	104.75	104.78	11.12
No	65	94.2	37.3-128.6	50.24	67.43	30.91
P	0.0352					

Presence of DCM significantly raises CA2M Values.

Table 14

Pulmonary hypertension and CA2M Values

H/O PHT	Cases		CA2M Values			
	No.	**%**	**Range**	**Median**	**Mean**	**S.D.**
Yes	15	21.7	87.28-128.6	108.27	110.84	16.14
No	54	78.3	37.3-127.02	64.28	58.14	23.99
p	0.0001					

HIV patients having PHT have significantly higher CA2M values.

As depicted in Table 1 and Table 2 all the four study groups are comparable with respect to age and sex .Similarly age and sex did not have any effect of CA2M values (Table 6and 7). There is statistically significant difference (p value <0.0001) between CA2M values of HIV patients having cardiac involvement (Group A , mean CA2M levels 98.99 mg/dl) and HIV patients without cardiac involvement(Group B , mean CA2M levels 45.6 mg/dl). The mean CA2M levels for normal healthy controls (Group D) was 36.94 mg/dl . Group C patients which comprised of patients with known cardiac illnesses had mean CA2M levels 125.46mg/dl. This high levels are accounted by the fact that majority of them had significant LV dysfunction on echocardiography disproportionate to clinical symptoms which were ameliorated by treatment.

The main HIV – associated cardiovascular abnormalities noted in this study were pericardial effusion, dilated cardiomyopathy and pulmonary hypertension. Two patients had left ventricular diastolic dysfunction. 4 patients had mitral valve prolapse, which though not related to HIV infection, was included as they had grade 1 to 2 MR. Two patients had grade 1 and 2 Aortic regurgitation respectively and they have also been included , but HIV-related infective endocarditis as a cause of aortic regurgitation was not proved by echocardiography. Among the

HIV-related complications pulmonary hypertension had the highest incidence (21.7%), followed by pericardial effusion (14.5 %) and dilated cardiomyopathy (5.8%). Eight patients had more than one lesion. Overall incidence of patients who were noted to have cardiac involvement both HIV-related and non-related was 41.2% . Subgroup analysis showed that presence of pericardial effusion , dilated cardiomyopathy and pulmonary hypertension had a significant rise in CA2M levels (p value <0.001) illustrating the correlation of CA2M levels with cardiovascular manifestations of AIDS.

DISCUSSION

The dawn of 21st century has witnessed the emergence of AIDS as the fourth leading cause of mortality worldwide[1]. Despite implementation of various awareness programs and containment strategies, the disease continues to spread at a tremendous pace and poses the biggest challenge the medical science faces today. With introduction of effective antiretroviral therapy, there was a significant change in the scenario as these therapies could improve the impaired host defense machinery. This reduced the incidence of fatal opportunistic infections and substantially improved the life expectancy of diseased. Follow up of these patients lead to the recognition of coexisting morbid pathologies involving multiple organ systems which were not adequately focused till then.

Involvement of cardiovascular system, either direct or indirect in AIDS has now been contemplated to be a major factor contributing to significant morbidity and mortality in patients[2-7].A wide spectrum of cardiac manifestations has now been recognized in AIDS. Notable among them are pericardial effusion, dilated cardiomyopathy , pulmonary hypertension, accelerated atherosclerosis related to antiretroviral therapy, myocarditis, vasculitis and cardiac neoplasms[2-7]. Recent studies highlighted the impact of these in the course of illness and strongly recommended an active pursuit of these complications in patient workup .

Hitherto available markers like CD4 counts and HIV RNA levels do not correlate with many of these manifestations including pericardial effusion[22] and pulmonary hypertension[6,27]. Techniques like echocardiography and angiography need involving specialized practitioners and is not a feasible option as it is cost ineffective and not available in all settings.

Currently available molecular markers including CK-MB, Troponin T/I etc are specific for myocardial infarction and not useful in other forms of cardiac involvement. BNP is an established marker for cardiac failure but its clinical utility is confined to distinction of acute pulmonary edema from respiratory causes of acute dyspnoea. Hence the identification of a new marker, which is uniformly elevated in all forms of cardiac illnesses irrespective of the primary pathologic process, would be a novel one in detection of AIDS patients having cardiac involvement who can be further managed in concert with specialists.

CA2M is a new molecular marker which may answer the search for a "universal" cardiac marker. It came to light first through the pioneer works by Rajamanickam et al[9]who isolated this molecule from the heart of experimental rats in which hypertrophy was induced by constriction of aorta. It was postulated that CA2M expression induce cardiac hypertrophy as its levels directly correlated with left ventricular mass

index[10-11].This was confirmed when injection of purified cDNA of CA2M induced cardiac hypertrophy upon direct injection into the heart of experimental rats[12]. Also it was observed that in vivo administration of polyclonal antibody raised against CA2M abolished cardiac hypertrophy by downregulating the expression of β-MHC and MLC-2 in experimental animals[12].Since hypertrophy is the early adaptive response of the failing myocardium , it was proposed that CA2M could be employed as a molecular marker , levels of which raises in cardiac dysfunction resulting from various pathologic processes. Its clinical utility was first tested by Dr Ratinavel (2005) et al [13]who observed that the levels of CA2M are significantly elevated in cardiac dysfunction resulting from different pathologies including ischemia , rheumatic heart disease , congenital heart disease etc irrespective of the primary pathology. Subsequent study by Annapoorni et al (2006)[14]established CA2M as a marker for detection of post myocardial infarction status in silent ischemia in diabetic individuals.

The present study was conducted with an aim of early identification of cardiovascular involvement in HIV infected individuals. A total of 69 HIV who had NYHA class I and II dyspnoea and who did not have any prior history of any cardiac ailments were analyzed for cardiovascular system involvement by echocardiography and

electrocardiography. Since the aim of the study was to analyze the usefulness of CA2M as an early marker for cardiac involvement in HIV, patients with clinical features of NYHA class III/IV cardiac failure and signs of failure like S3 gallop, elevated JVP and lung basal crepitations were not included in the study. The main cardiovascular abnormalities noted in this study were pericardial effusion, dilated cardiomyopathy , pulmonary hypertension and left ventricular diastolic dysfunction. Overall incidence of patients who were noted to have cardiac involvement both HIV-related and non-related was 41.2%. The non-HIV related cardiac manifestations included mitral valve prolapse with grade1-2 mitral regurgitation and mild aortic regurgitation. But they were also taken into account as their CA2M levels differed significantly from those without any cardiac involvement in echocardiography. The higher incidence of cardiac involvement in the study compared to other large scale clinical and autopsy studies may be owing to this and also due to cardiac symptom-based screening of HIV patients.

Pericarditis and pericardial effusion constitute most common recognized cardiac involvement in HIV infection [2-7,22]. In a review of 15 autopsy and echocardiographic series involving 1139 patients[22] with HIV disease, incidence of pericardial disease was 21%. Incidence of pericardial effusion in my study was 14.5 %. Factors causing

pericardial effusion include opportunistic infections, most common being mycobacterium tuberculosis or MAI infection , direct viral toxicity and malignancies - Kaposi's sarcoma and lymphoma. It may a part of a generalized capillary leak syndrome [22,23] due to overproduction of inflammatory cytokines. Others are uremia and drug toxicity. Occurrence of pericardial effusion is independent of CD4+ count and is a grim prognostic sign[22]. The cause of pericardial effusion was not analyzed due to constraints in facilities for pericardial fluid analysis .Different grades of effusion ranging from mild effusion to severe including one case of effusive constrictive pericardial effusion (CA2M level- 127.02 mg/dl) were observed. Mean values of CA2M levels for pericardial effusion was 114.73±14.7 (S.D) mg/dl (table 12) which is significantly higher compared to HIV infected patients without cardiac involvement (45.6±5.8 mg/dl)(table 3) and normal healthy individuals (36.94±2.6 mg/dl) (table 3).

Ventricular dysfunction due to dilated cardiomyopathy is another important cardiac manifestation in HIV infection. A 4 year observational study by Currie PF et al. 296 patients with HIV infection revealed 15% have dilated cardiomyopathy, 13 % isolated right ventricular dysfunction and 12 % with borderline left ventricular dysfunction[5]. In the present study incidence of dilated cardiomyopathy was 5.8% . All

patients had only mild to moderate LV dysfunction. Postulated mechanisms for HIV – mediated cardiomyopathy include direct cardiotoxicity of HIV virus, opportunistic infections, autoimmune response and nutritional deficiencies. Though exact mechanism remains elusive majority consider it to be result of postmyocarditis cardiomyopathy. Idiopathic lymphocytic myocarditis is a common postmortem finding in patients with ventricular dysfunction The overexpression of cytokines TNF-α , IL s and inducible nitric oxide synthase (INOS) evident in endomyocardial biopsies appears to be equally responsible. Occurrence of cardiomyopathy in children , in whom a disease unrelated to HIV infection would be rare , suggests a direct relationship between HIV disease and cardiomyopathy. Presence of cardiomyopathy correlate with low CD4+ counts and is a grave prognostic mark [18-23]. Mean values of CA2M levels for dilated cardiomyopathy was 104.78 ± 11.12 mg/dl (table 13), significantly elevated compared to HIV patients without cardiac involvement and normal healthy individuals.

2 independent analysis by Saeoane et al. and Saide et al. in hospitalized AIDS patients, primary pulmonary hypertension is estimated to occur in about 0.5% [24-27]. In my study pulmonary hypertension had the highest incidence (21.7%) among the observed HIV - associated cardiac illnesses. Majority had only mild pulmonary

hypertension . The difference between incidence in other studies in AIDS patients and this study group is noteworthy. Since large-scale studies in this area is lacking in Indian population influence of racial difference can not be attributed. Small size of the present study population is also a hindrance in assessing whether this was a mere coincidence or a significant difference. Further large-scale analysis is needed for clarification of this issue. Lung histology frequently demonstrates plexogenic arteriopathy as in other cases of primary pulmonary hypertension. It is often considered to develop secondary to recurrent lung infections, venous thromboembolism or left ventricular dysfunction but its occurrence in HIV-infected patients without documentation of prior history of any of these risk factors indicate some hitherto elusive mechanisms also contribute. Hypothesized factors are cytokine related stimulation and proliferation of endothelium and smooth muscle hyperplasia due to receptor mediated action of viral proteins. In a review of 88 cases Mesa et al. found no correlation of pulmonary hypertension with CD4 count[27]. Mean values of CA2M levels for pulmonary hypertension was 110.84±16.14 mg/dl (table 14), significantly elevated compared to HIV patients without cardiac involvement and normal healthy individuals.

To compare the results with the general population 10 patients with known cardiac illness including RHD, congenital heart disease and

ischemic heart disease and 10 normal individuals were also simultaneously analyzed. Results were as follows :

Group C - Patients with cardiac illnesses had a mean CA2M value 125.46 $\pm$ 9.07 mg / dl and Group D - Patients with cardiac illnesses had a mean CA2M Value 36.94 $\pm$ 2.96 mg/dl. These results are comparable with that of the study conducted by Dr.Ratanavel et. al.,[14] (cardiac disease 131 $\pm$ 27.14 mg/dl and Normal 46 $\pm$ 6 mg/dl) who demonstrated a significant elevation of CA2M levels in patients with cardiac dysfunction resulting from various cardiac diseases.

Overall analysis of the data clearly indicates that CA2M levels rise significantly with all forms of cardiac involvement in HIV patients irrespective of the nature of the primary illness. This elucidates the role of CA2M as a molecular marker for cardiac involvement in HIV-infected patients . Since the study population comprised only of patients having mild to moderate symptoms and echocardiographic findings consistent with mild to moderate cardiac dysfunction it can be concluded that CA2M may be utilized for early detection of cardiac involvement in HIV-infected patients. This may enable physicians in categorizing the HIV-infected patients for effective management and also in prognosticating the disease as studies have highlighted the fact that cardiac involvement correlates with a bad prognosis in the course of the illness[2-5,18].

Limitations of the study

Since the study population was small large-scale studies may be required to explore the profound utility of this biomarker in clinical practice. Another hindrance at present is the high cost involved in the manufacturing of the antisera as this molecule is still in the experimental phase. But if the efficacy is confirmed by large-scale analysis and a definite indication is formulated then this can be overcome by involving multinational biotechnology manufacturing sectors. As the study was a cross-sectional analysis with all tests done in one sitting, follow up of patients could not be performed. This disallowed from assessing the alterations of CA2M levels with HAART therapy. Hence a definite prognostic role besides that as a cardiac marker could not be established. Further follow up studies are warranted in this aspect. Similarly due to non -availability of a protease inhibitor based HAART regimen therapy induced lipid abnormalities and accelerated atherosclerotic diseases were not analyzed in this study.

CONCLUSION

1. CA2M levels are significantly elevated in HIV patients with cardiac involvement.
2. CA2M can be utilized as an early molecular marker in HIV infected individuals for assessing cardiac involvement who do not have symptoms or signs of overt congestive cardiac failure.

SUMMARY

The present study was undertaken with an aim to establish the diagnostic utility of CA2M as an early biomarker for cardiac involvement in HIV infected individuals. A total of 69 HIV infected patients with history suggestive of cardiac involvement were analyzed, after excluding patients with known history of other cardiac illnesses and patients with advanced symptoms. On echocardiographic evaluation 31 had cardiac involvement. Main abnormalities noted in my study were pulmonary hypertension, pericardial effusion, dilated cardiomyopathy and LV diastolic dysfunction. For comparison of results with a general population 10 patients with known cardiac diseases and 10 normal control individuals were also analyzed.

CA2M levels were found to be significantly elevated in HIV patients who had cardiac involvement and values were comparable to patients without HIV infection who had cardiac illnesses as noted in a previous study by Dr Ratinavel et al. This clearly illustrates the role of CA2M as an early biomarker for cardiac involvement in HIV. Along side it was also observed that pulmonary hypertension had a higher incidence in the analyzed HIV-infected population which has not so far been reported in any other studies conducted to date.

APPENDIX - I

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APPENDIX - II

PRO FORMA

CARDIAC ISOFORM OFALPHA-2-MACROGLOBULIN AS AN EARLY MARKER FOR CARDIAC INVOLVEMENT IN HIV/AIDS PATIENTS

NAME: AGE: SEX:

IP No/ADDRESS: OCCUPATION:

SYMPTOMS:

CVS: h/o dyspnoea/orthopnoea/PND :

h/o angina/angina equivalents :

h/o dependent edema :

HIV related: h/o weight loss :

h/o chronic fever/cough :

h/o chronic diarrhoea :

h/o skin manifestations :

others :

PAST HISTORY

HT: DM: IHD: POVD:

STROKE/TIA :

H/O PULMONARY TUBERCULOSIS :

IF YES H/O ATT INTAKE :

HIV STATUS

DURATION OF ILLNESS :

CD4 COUNT AT DIAGNOSIS :

H/O OPPORTUNISTIC INFECTIONS :

DURATION OF ANTIRETROVIRAL THERAPY:

ART DRUGS TAKEN :

PERSONAL HISTORY

SMOKING/TOBACCO : **ALCOHOL:**

FAMILY HISTORY

HT: **DM:** **IHD:**

O/E:-

PR- **BP-** **CAROTIDS-** **JVP-**

PERIPHERAL VASCULAR SYSTEM-

CVS:-

APICAL IMPULSE SITE(ICS): **CHARACTER:**

S1 **S2** **S3** **S4**

MURMUR-SYST/DAIST : **AREA :**

E/O EFFUSION :

RS:-

P/A:-

CNS:-

INVESTIGATIONS

Hb(g%): TC(per mm^3): DC(%):P L E M

RBS(mg/dl): B Urea(mg/dl): S Creat(mg/dl):

S LIPID profile -

T Cholesterol - LDL - VLDL -

HDL - T Triglycerides-

CD count: CD4 (per mm^3)-

CD8 (per mm^3)-

CD4 / CD8 RATIO-

HIV STATUS

ELISA-

WESTERN BLOT-

CXR PA VIEW

ECG- RATE SR/NON SR P wave

PR interval QRS ST-T segment-

ECHO- LVID(S) LVID(d) EF

MV- AV- TV- PV-

PERICARDIAL EFFUSION-

MV E/A- LVOT-

PULMONARY HYPERTENSION-

CARDIAC ALPHA-2 MACROGLOBULIN (CA2M) LEVELS-

APPENDIX - III

ABBREVIATIONS

ANF	-	Atrial Natriuretic Factor
ATP	-	Adenosine tri phosphate
BNP	-	Brain natriuretic peptide
cDNA	-	Complementary deoxyribonucleic acid
dNTP	-	Deoxynucleotide triphosphate
DCM	-	Dilated Cardiomyopathy
EDTA	-	Ethylene diamine tetraacetic acid
g, mg, µg, ng-		gram, milligram, microgram, nanogram
HPLC	-	High performance liquid chromatography
IgG	-	Immunoglobulin – G
IL	-	Interleukin
INOS	-	Inducible nitric oxide synthase
MHC	-	Myosin heavy chain
MLC	-	Myosin light chain
mRNA	-	messenger ribonucleic acid
PCR	-	Polymerase chain reaction

PMSF	-	Phenyl methyl sulphonyl fluoride
PKC	-	Protein kinase C
PHT	-	Pulmonary Hypertension
RHD	-	Rheumatic Heart Disease
TGF-β	-	Transforming growth factor – β
TNF -α	-	Tumor Necrosis Factor - α

Fig. 10a: Chest X-Ray showing pericardial effusion in a HIV patient

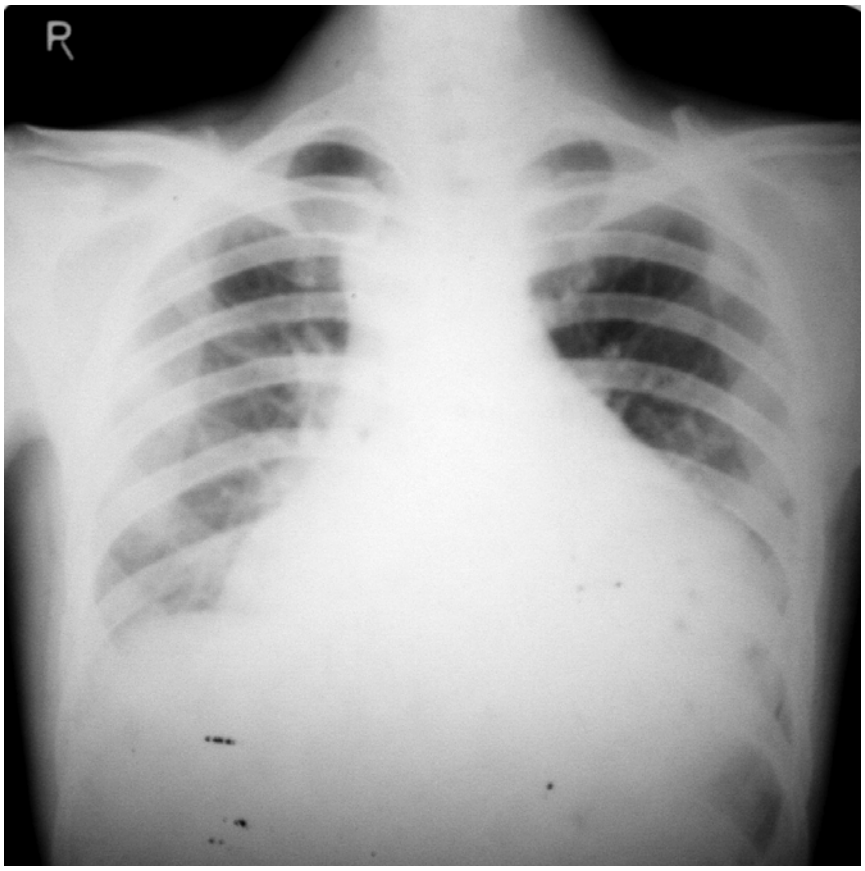


Fig. 10b: 2-D Echo showing posterior echo-free space indicating pericardial effusion

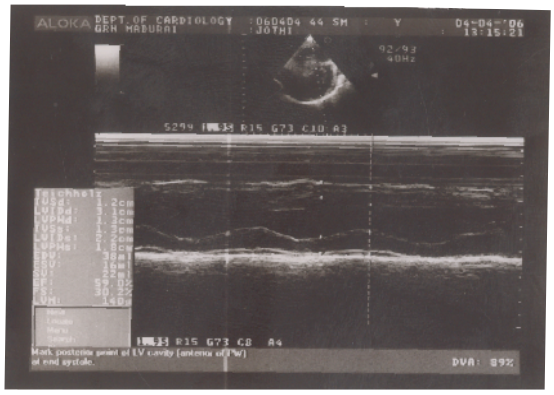


Fig. 11a: M-mode Echo showing dilated ca rdiomyopathy

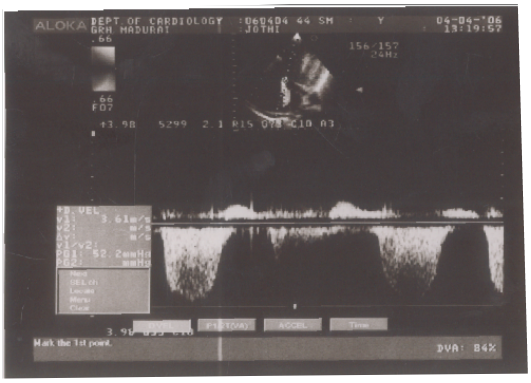


Fig. 11b : Doppler Echo showing mitral regurgitation

Fig. 3 AGE COMPOSITION OF STUDY POPULATION

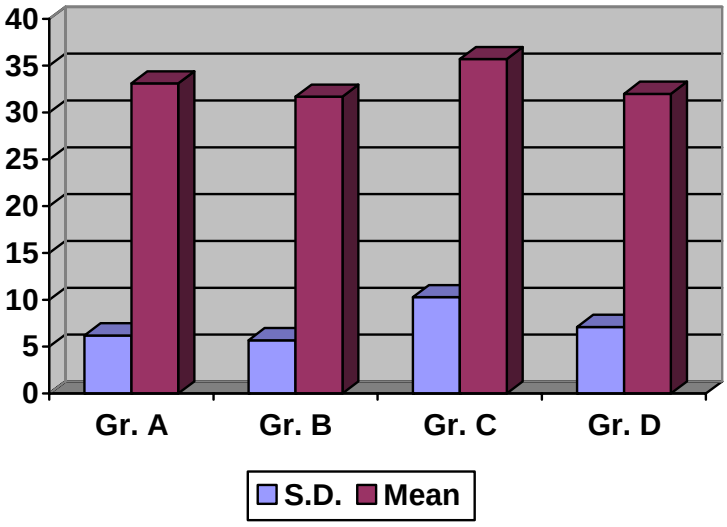
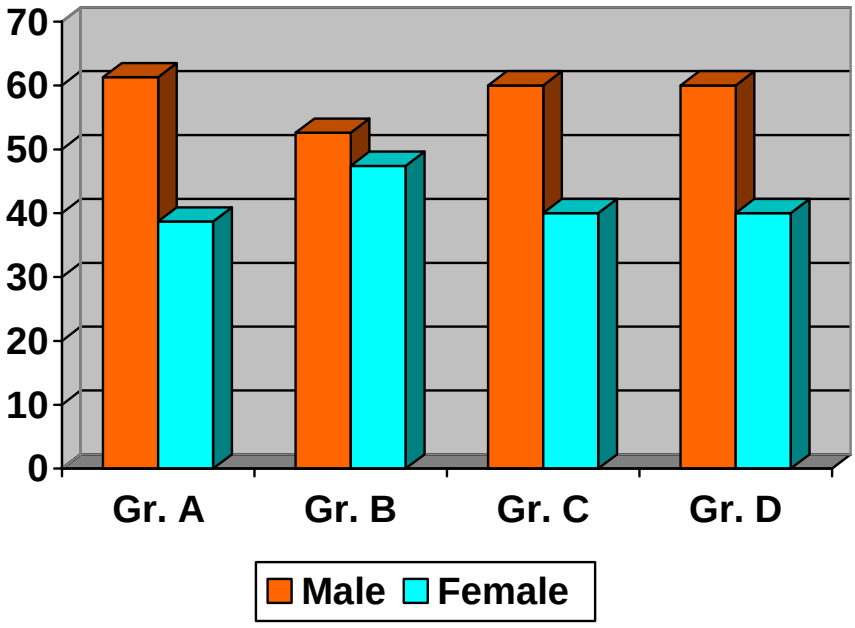


Fig. 4 SEX COMPOSITION OF STUDY POPULATION



CA2M Levels (mg/dl) in various Groups

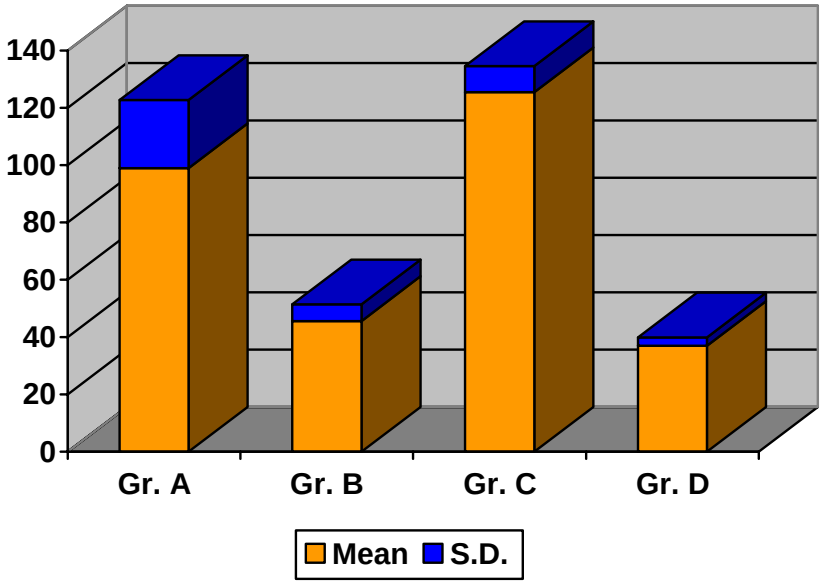


Fig 6 : **Pie chart of echocardiographic analysis of study groups A and B.**

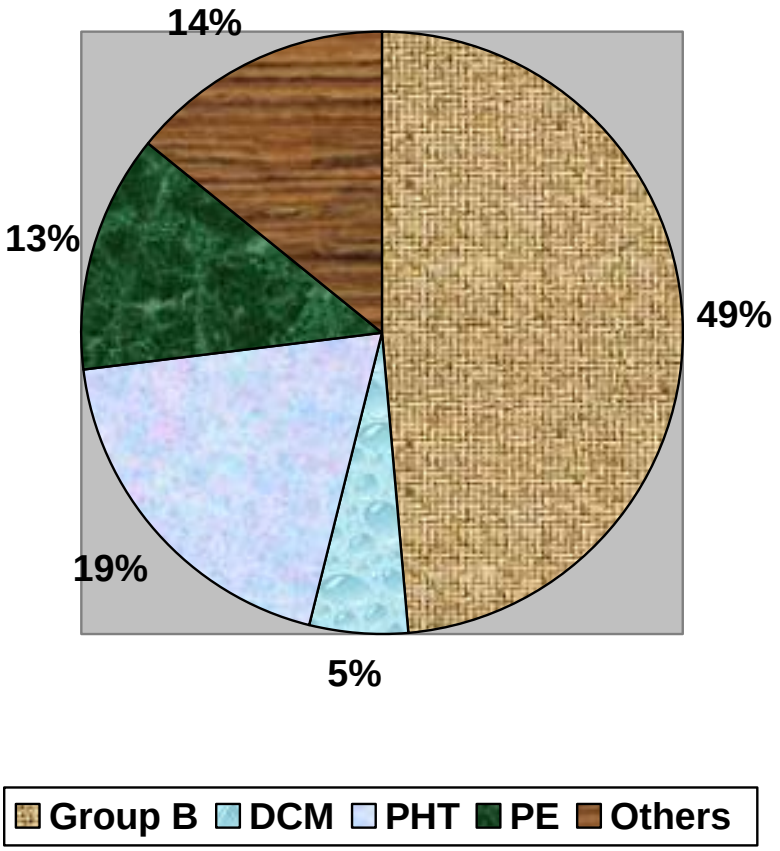


Fig. 7 PERICARDIAL EFFUSION AND CA2M VALUES

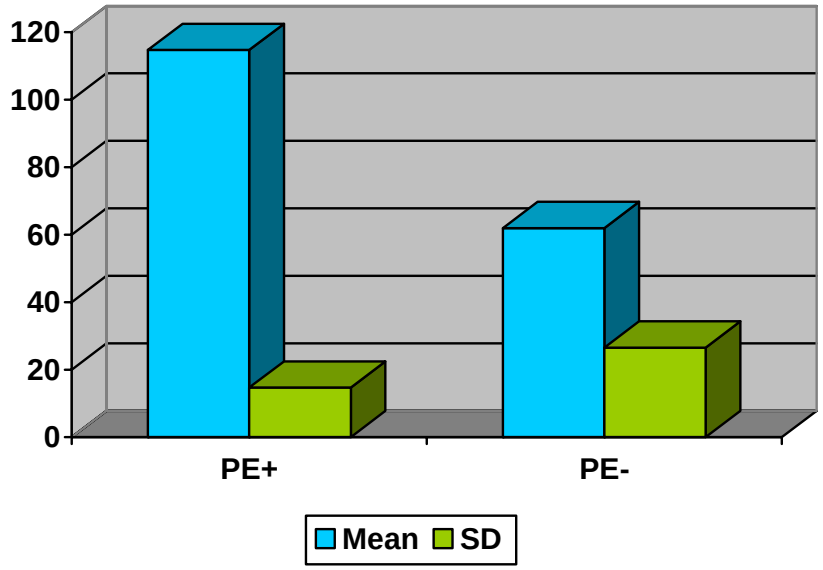


FIG. 8. DILATED CARDIOMYOPATHY AND CA2M VALUES

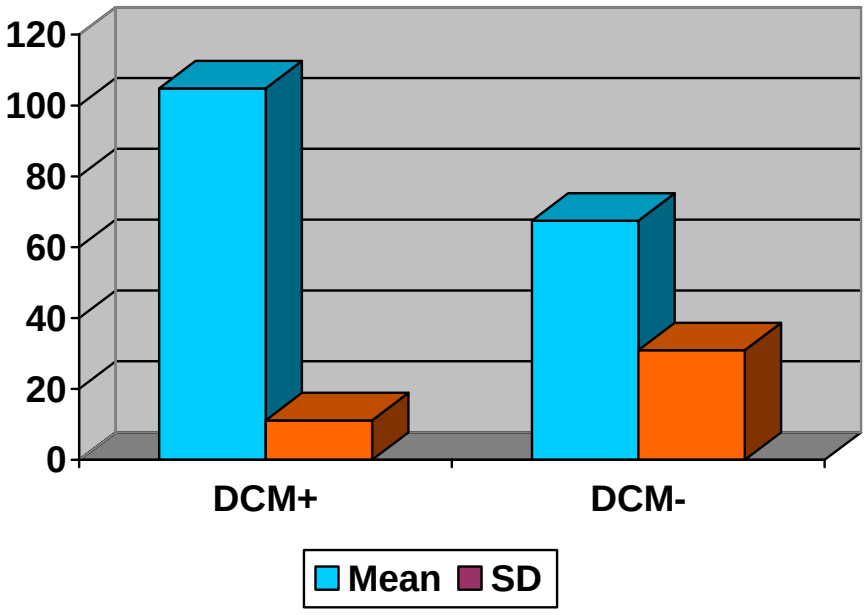


FIG. 9 PULMONARY HYPERTENSION AND CA2M VALUES

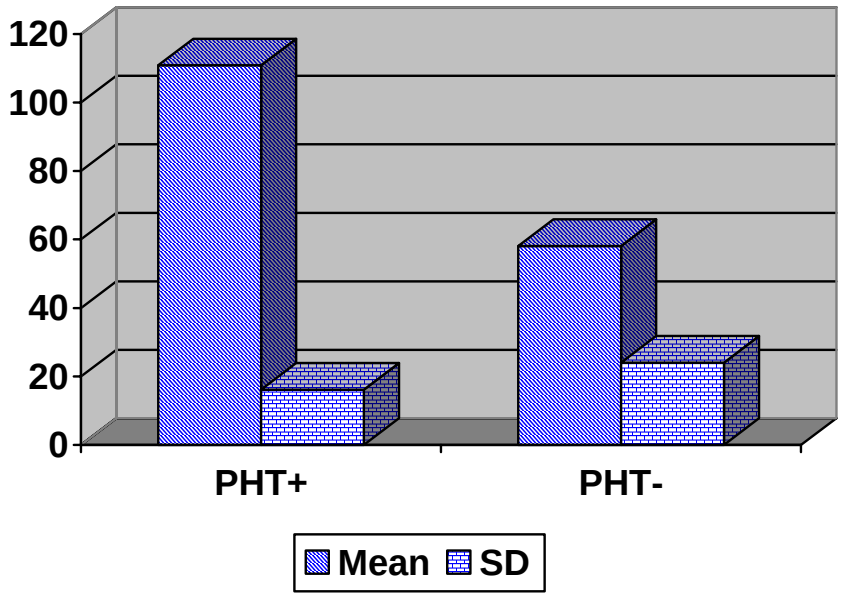
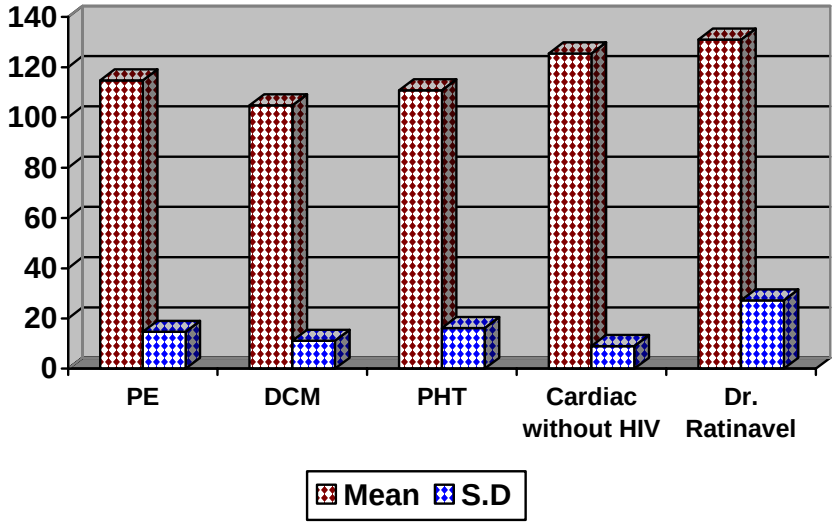


FIG. 12. CA2M LEVELS AND CARDIAC INVOLVEMENT

COMPARISON TO STUDY BY Dr. RATINAVEL



APPENDIX IV - MASTER CHART

HIV patients – Group A and B

SL. NO	AGE	SEX	CARDIAC SYMPTOM	DURATION (YEARS)	CD4 COUNT/mm^3	ART (Y/N)	H/O pulTB	PE	DCM	PHT	OTHERS	CAZM (mg/dl)
1	35	F	Y	1.5	178	ZLN	-	-	-	-	-	58.18
2	42	M	Y	1	30	SLN	+	-	-	+(Mod)	-	97.42
3	28	M	Y	3	165	SLN	+	-	-	-	-	49.86
4	25	F	Y	2	134	SLN	+	-	-	-	-	59.65
5	28	M	Y	1	72	SLN	+	-	+(Mild Lvdysf)	-	-	91.34
6	32	M	Y	1	70	SLN	+	+(eff constr)	-	-	-	127.02
7	37	M	Y	3	45	ZLN	-	+ (modPE)	-	+(sev)	-	128.01
8	24	F	Y	3.5	76	SLN	-	+(mild PE)	-	+(mild)	-	108.21
9	45	M	Y	10	216	ZLN	-	-	-	-	-	54.01
10	31	M	Y	1	90	ZLN	-	+(mild PE)	-	+(mild)	-	96.86
11	36	M	Y	4	93	SLN	+	-	-	-	Grade 2 Aortic Regurgitation	92.29
12	22	M	Y	1	203	SLN	-	-	-	-	-	41.67
13	22	F	Y	2	174	ZLN	-	-	-	-	Mitral valve prolapse / MR Grade 1	64.28
14	28	F	Y	3	228	ZLN	-	-	-	+(mild)	Trivial mitral regurgitation	87.28
15	33	F	Y	2	143	ZLN	+	-	-	-	-	59.45
16	34	F	Y	1.5	138	SLN	+	-	-	+(mild)	-	89.16

SL. NO	AGE	SEX	CARDIAC SYMPTOM	DURATION (YEARS)	CD4 COUNT/mm^3	ART (Y/N)	H/O pulTB	PE	DCM	PHT	OTHERS	CAZM (mg/dl)
17	36	M	Y	2	152	SLN	-	-	-	-	Trivial mitral regurgitation	42.57
18	28	M	Y	4	186	SLN	-	-	-	-	-	42.4
19	25	F	Y	2	268	SLN	-	-	-	+(mild)	-	106.02
20	29	F	Y	0.5	106	SLN	+	-	-	+(mild)	Mitral valve prolapse Grade 2 MR	118.24
21	43	M	Y	2	59	ZLN	-	-	-	+(mild)	-	128.49
22	30	F	Y	1	60	ZLN	-	-	-	+(mod)	-	108.27
23	26	M	Y	1	78	SLN	-	-	-	-	-	43.56
24	38	M	Y	2	78	ZLN	+	-	-	-	-	50.24
25	33	M	Y	3	128	SLN	+	-	-	-	-	43.53
26	30	F	Y	3	100	SLN	+	-	-	-	-	42.72
27	35	M	Y	1	142	ZLN	-	-	-	-	-	46.81
28	29	F	Y	1	205	ZLN	-	-	-	-	-	39.36
29	21	F	Y	2	176	SLN	+	-	-	-	-	50.36
30	32	F	Y	8	155	SLN	-	-	-	-	-	40.31
31	24	F	Y	2	98	SLN	+	-	-	-	-	38.4
32	28	F	Y	1	90	ZLN	-	-	-	-	-	42.9
33	36	F	Y	1	165	ZLN	+	-	-	-	-	46.4
34	33	F	Y	1	175	ZLN	-	-	-	-	-	37.3
35	45	M	Y	1	110	ZLN	-	-	-	+(mod)	Grade 2 Mitral Regurgitation	128.6
36	34	M	Y	1	112	SLN	+	-	-	-	-	44.69
37	28	M	Y	1	95	ZLN	-	-	-	-	-	40.6
38	41	M	Y	1	110	ZLN	-	-	-	-	LV diastolic dysfunction	90.16
39	29	F	Y	1	50	ZLN	-	-	-	-	-	43.8
40	35	M	Y	4	50	ZLN	+	+(mod PE)	-	+(mild)	-	124.01
41	37	M	Y	2	160	SLN	+	-	-	-	-	51.46
42	28	F	Y	3.5	102	SLN	-	-	-	-	-	42.08

SL. NO	AGE	SEX	CARDIAC SYMPTOM	DURATION (YEARS)	CD4 COUNT/mm^3	ART (Y/N)	H/O pulTB	PE	DCM	PHT	OTHERS	CAZM (mg/dl)
43	34	M	Y	4	46	ZLN	-	-	+Gr2LV sys dysf	-	-	118.26
44	46	M	Y	3	66	SLN	+	+(mild PE)	-	+(mild)	-	128.01
45	31	M	Y	2	60	SLN	+	-	-	-	-	46.24
46	25	F	Y	1.5	70	ZLN	-	-	-	-	-	40.82
47	35	M	Y	2	95	SLN	+	-	-	-	-	44.69
48	29	M	Y	1	105	ZLN	-	-	-	-	-	42.08
49	29	F	Y	2	180	ZLN	-	-	-	-	Mitral valve prolapse Grade 1 MR	50.06
50	40	M	Y	6	160	SLN	+	-	-	-	-	46.48
51	33	F	Y	3	138	ZLN	-	-	-	-	-	49.38
52	38	M	Y	2	152	ZLN	+	-	-	-	-	52.36
53	39	M	Y	1	188	SLN	+	-	-	-	-	40.42
54	26	F	Y	2	270	ZLN	+	-	-	-	Mitral valve prolapse Grade 1 MR	68.38
55	40	M	Y	4	120	SLN	-	+(mild PE)	-	-	-	92.38
56	27	F	Y	5	70	SLN	-	+(mod PE)	-	-	-	120.34
57	34	M	Y	8	60	ZLN	-	+(mod PE)	-	+(mild)	-	126.21
58	39	M	Y	2	105	ZLN	+	-	-	-	-	48.82
59	29	M	Y	6	130	SLN	-	-	-	-	Grade 1 AR	70.82
60	34	M	Y	3	140	ZLN	-	-	+(LV sys dys)	-	-	102.68
61	26	F	Y	2	210	ZLN	+	-	-	-	-	42.82
62	29	F	Y	1	170	ZLN	-	-	-	-	-	48.24
63	43	M	Y	3	160	ZLN	-	-	-	-	-	40.46
64	30	F	Y	2	100	SLN	+	-	-	-	LV Grade 1 Diastolic dysfunction	72.46

SL. NO	AGE	SEX	CARDIAC SYMPTOM	DURATION (YEARS)	CD4 COUNT/mm^3	ART (Y/N)	H/O pulTB	PE	DCM	PHT	OTHERS	CAZM (mg/dl)
65	36	M	Y	2	92	ZLN	-	-	-	+(mild)	-	87.86
66	28	F	Y	4	48	SLN	-	+(mod PE)	-	-	-	96.24
67	33	F	Y	2	98	ZLN	+	-	-	-	-	38.42
68	35	M	Y	0.5	174	SLN	-	-	+(LV sys dys)	-	-	106.82
69	29	M	Y	2	230	SLN	-	-	-	-	-	42.42

Cardiac patients without HIV – Group C

SL NO	AGE	SEX	CARDIAC DISEASE	CA2M(mg/dl)
1	22	F	RHD/MR/PHT/CCF	128.28
2	30	F	RHD/MS/MR/PHT/CCF	136.42
3	46	M	Bicuspid Aortic Valve / AS / AR	122.42
4	47	M	CAHD / A/C AWMI/ LVEF – 58%	128.16
5	35	M	Dilated Cardiomyopathy / CCF	140.28
6	39	M	Pericardial Effusion (Tuberculosis)	122.14
7	20	F	VSD / PHT	130.21
8	28	M	OS - ASD	110.38
9	42	M	CAHD / OLD IWMI/ LVEF - 60%	120.16
10	48	F	CAHD / OLD IWMI/ LVEF - 66%	116.14

Normal individuals –Group D

SL NO	AGE	SEX	NORMAL CONTROLS	CA2M(mg/dl)
1	22	M	Normal Echo	40.38
2	30	F	Normal Echo	38.18
3	35	M	Normal Echo	34.12
4	40	F	Normal Echo	36.18
5	28	M	Normal Echo	38.21
6	19	F	Normal Echo	36.18
7	40	M	Normal Echo	34.28
8	35	M	Normal Echo	37.18
9	36	M	Normal Echo	32.46
10	35	F	Normal Echo	42.18